Amendments

Amendments to the Specification

Please replace the paragraph starting on page 3, line 36 and ending on page 4, line 2 with the following paragraph:

B1

The "extract" or "component" is an extract or component that is immunogenic such that antibodies raised ~~aginast~~ against the extract or component of a commensal *Neisseria* cross react with a pathogenic *Neisseria*, in particular *N. meningitidis*.

Please replace the paragraph on page 14, line 13, with the following paragraph:

B2

Fig. 7 is a map of Neisserial shuttle plasmid pMIDG100 containing a multiple cloning site (SEQ ID Nos: 13 and 14);

Please replace the paragraph on page 14, lines 22-24, with the following paragraph:

B3

Fig. 10 shows a plasmid map of pMIDG201, containing a kanamycin cassette, a *rho*-independent upstream transcriptional terminator (SEQ ID NO: 15), a multiple cloning site (SEQ ID NO: 16) and the ner promoter; and

Please replace the paragraphs starting on page 17, line 14 and ending on page 18, line 4 with the following paragraphs:

B4

Brain heart infusion agar plates were inoculated with 50µl of *N. lactamica* strain Y92-1009 and incubated overnight at 37°C, with 5% CO_2. This was used to inoculate a 100ml MHB starter culture which was incubated with shaking at 37°C for 6 h. Starter culture (15ml)

was added to each of 6x500ml volumes of MHB. These were then incubated with shaking overnight at 37°C and the conditions were made iron-limited by the addition of ~~5ugml^{-1}~~ 5μgml^{-1} EDDHA. The cells were harvested by centrifugation and the supernatant discarded. The cells were washed with 100ml PBS and then pelleted by centrifugation. Cell pellets were resuspended in PBS + 0.3% (v/v) ~~Elugent~~ ELUGENT® (Calbiochem, 2ml per g wet weight), an eluting reagent, and incubated with shaking at 37°C for 20 min. The cells were then removed by centrifugation and the pellet discarded. EDTA and N-lauroyl sarcosine were then added to the supernatant to 10mM and 0.5% (w/v) respectively.

An electrophoresis unit, ~~The~~ the BioRad® ~~(Registered Trade Mark)~~ Prep Cell, model 491 was then used to separate the proteins contained in the detergent extract. A 4cm, 7% acrylamide native resolving gel was cast with a 2 cm stacking gel. 12mg of protein in native sample buffer was electrophoresed using running buffer containing 0.1% (w/v) SDS, 0.025M Tris and 0.192M glycine at 40mA and 400V until the dye front reached the bottom of the gel. 3ml fractions of the eluted proteins were then collected. Once the fractions were collected they were pooled into groups consisting of proteins of molecular weight approximately less than 40kDa, between 40 and 67kDa and more than 67kDa. The pooled proteins were concentrated by ammonium sulphate precipitation and dialysed against PBS. These were diluted in PBS to a protein concentration of 100ug/ml and Freund's complete adjuvant was added at a ratio of 1:1(v/v) or Freund's incomplete adjuvant for booster doses.

Please replace the paragraph on page 18, lines 15-19, with the following paragraph:

Mice were vaccinated with no vaccine (i.e. control group), ~~Elugent~~ ELUGENT® ~~("Registered Trade Mark")~~ extract or high, medium or low molecular weight fraction. The

B6 mice receiving the protein fraction groups received 0.2ml subcutaneously; equivalent to 10μg of protein.

Please replace the paragraph on page 20, lines 26-27, with the following paragraph:

B7 In this series of examples, we have expressed two ~~of~~ foreign antigens, GFP and NspA, in a range of commensal *Neisseria*.

Please replace the paragraphs starting on page 25, line 24 and ending on page 26, line 3 with the following paragraphs:

E. coli S17-1 1 *pir* donor strains, containing plasmid, were grown overnight in LB broth containing 75 μg/ml kanamycin, then diluted 1:100 in sMH broth with no antibiotics, and incubated without shaking for 1.5 hours at 37°C in 5% CO_2. Overnight plates of the recipient nalidixic acid resistant Neisseria strains were harvested into 5 ml of pre-warmed sMH broth (approximately 10^8 CFU/ml) and diluted 1:50 in 50 ml of pre-warmed sMH broth. This subculture was incubated without shaking for 2 to 4 hours at 37°C with 5% CO2 in a 50ml flask which allows a high surface to volume ratio. 300 μl recipient and 50 μl donor

B8 cells from these subcultures were pipetted onto a 0.45 μM pore size membrane filter (~~Millipore~~ MILLIPORE®) and placed on a sGC agar plate, which were then incubated overnight. The filters were scraped and/or vortexed into 5 ml of MH broth in a 50 ml skirted tube. The filter was removed and the suspension was centrifuged at 3000 g for 10 min. The pellet was resuspended in 100 to 200 μl of residual media before being plated onto GC plates containing 20 μg/ml nalidixic acid and 75 μg/ml kanamycin. These plates were incubated overnight and single colonies selected for further evaluation.

Please replace the paragraph on page 26, lines 30-34, with the following paragraph:

B9

Plasmid DNA was purified from *E. coli* host cells using QIA Spin® plasmid kit or ~~Qiagen~~ QIAGEN® plasmid midi kit according to the manufacturers instructions (Qiagen, UK). The protocols were based on a modified alkaline lysis procedure followed by binding plasmid DNA to a ~~Qiagen~~ QIAGEN® anion-exchange resin under appropriate low salt and pH conditions.

Please replace the paragraph on page 27, lines 1-4, with the following paragraph:

B10

Neisseria strains were grown to confluence on sGC agar plates with appropriate antibiotics when necessary. The bacteria were harvested from three plates and plasmid DNA was purified using the ~~Qiagen~~ QIAGEN® plasmid midi kit according to the manufacturers instructions (Qiagen, UK).

Please replace the paragraph on page 27, lines 8-11, with the following paragraph:

B11

Neisseria strains were grown to confluence on GC agar plates with appropriate antibiotics when necessary. The bacteria were harvested from a single plate and chromosomal DNA isolated using the QIAGEN® Genomic-tip System according to the manufacturers instructions (Qiagen, UK).

Please replace the paragraph on page 27, lines 15-19, with the following paragraph:

B12

DNA fragments were excised from agarose gels, to which ethidium bromide was added, using a scalpel blade whilst DNA was visualised using a UV transilluminator (302nm). DNA was purified from the agarose gel using a QIAquick® gel extraction kit according to the manufacturers instructions (Qiagen, UK).

Please replace the paragraph on page 28, lines 5-9, with the following paragraph:

PCR reactions consisted of DNA, 1 pmol/µl of each primer (Table 2.1), 0.2 mM dNTPs (deoxyribonucleoside triphosphates), 0.5 units HotStar® *Taq* DNA polymerase (Qiagen, UK), and PCR buffer with a final concentration of 100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$. Reactions were performed in a programmable heating block (Perkin Elmer).

Please replace the paragraph on page 28, lines 27-30, with the following paragraph:

Colony PCR using ~~Hybaid~~ HYBAID® recovery amplification reagent (Hybaid Ltd, UK) was carried out according to the manufacturers instructions. The Hybaid reagent works by sequestering cell lysis products, which may inhibit polymerases and improves amplification yield and specificity.

Please replace the paragraph on page 30, lines 24-26, with the following paragraph:

SDS-PAGE was performed as described in (Sambrook, 1989) using Protogel (BioRad™®) 30% acrylamide/ bis solution, 37.5:1 (2.6%C) and BioRad® Protean II kit. The gels were run at 25 mA per gel.

Please replace the paragraph on page 32, lines 13-19, with the following paragraph:

The fluorescent signal from Neisserial strains expressing GFP was quantified using a FACS Calibur flow cytometer (Becton-Dickinson) running ~~Cellquest~~ CELLQUEST® software (Becton-Dickinson). The flow cytometer settings used were as follows: FSC (channel EO2, log scale), SSC (log scale, amplification = 566, threshold =398), FL1-H (log scale, amplification = 674), and FL3-H (log scale, amplification = 705). Low flow rates were used for the evaluation of all samples.

Please replace the paragraph starting on page 33, line 36, and ending on page 34, line 2 with the following paragraph:

B17

Two clones for each conjugation positive strain were selected, one containing pMIDG100 the other pMIDG101, and a ~~Qiagen~~ QIAGEN® midi-prep was carried out to re-isolate the plasmid to ensure the plasmid had not inserted into the chromosomal DNA. Restriction digests verified the presence of intact pMIDG100 and pMIDG101.

Please replace the paragraph starting on page 36, line ~~36~~ 32, and ending on page 37, line 4 with the following paragraph:

B18

DNA was isolated from pMIDG101, pMIDG102, pMIDG103 and pMIDG104 and digested with *Bam*HI and *Xba*I to remove the *gfp* gene and transcriptional terminator however leaving the cloned promoters, which are present, intact. The fragment containing the pMIDG100 backbone and promoter was purified by agarose gel electrophoresis using a ~~Qiagen~~ QIAGEN® gel extraction ~~kit.~~ kit. Single stranded DNA oligonucleotides corresponding to the two strands of the sequence shown below were synthesised.

Please replace the paragraph on page 37, lines 19-36 with the following paragraph:

B19

These oligonucleotides (SEQ ID NOS: 9 and 10) were annealed by boiling at 100^{0}C for 5 min in ligase buffer and cooling to 4^{0}C. Initial attempts at cloning the linker directly into the pMIDG100 backbone were unsuccessful. The linker was ligated to pUC19 digested with *Bam*HI and *Xba*I and the ligation mix transformed into chemically competent *E. coli* TOP 10 cells. Two clones were selected. Plasmid was purified and sequenced using vector specific primer M13F. One clone was found to have the expected linker sequence confirming that the *Bam*HI and *Xba*I sites were intact. The linker was cut out of pUC19, separated from the vector, and ligated to the backbone of the pMIDG100 series of vectors.

The ligation mix was transformed into chemically competent *E. coli* TOP 10 cells. Twelve transformants of each construct were screened by colony blots, probing with DIG labelled linker: approximately 10 clones were shown to hybridise to the linker. Two clones containing each plasmid construct were ~~Qiagen~~ QIAGEN® ~~purified ,~~ purified, analysed by restriction digest and one clone was selected for sequencing. This confirmed that the sequenced clones were the desired construct and these were called pMIDG201 (*ner* promoter), pMIDG202 (*frp*C promoter), pMIDG203 (*rec*A promoter), and pMIDG204 (*gro*ES/EL promoter) respectively.

B19

Please replace the paragraph on page 39, lines 26-29, with the following paragraph:

To screen for expression of NspA in commensal Neisseria, a dot blot was carried out using Me-7 NspA monoclonal antibody (as described in the publication of Cadieux et al.[[, 1999]] (1999 Sep.) *Infect. Immun.* **67(9)**: 4955-9) on the following, using the A and B cassettes and promoters as set out:-

B20

Please replace the current sequence listing with the sequence listing attached hereto.